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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

AUG 20 1987

MEMORANDUM

SUBJECT: Notes of a meeting with the Office of Toxic Substances to discuss toxicity studies with tributyltin chemicals.

TOX CHEM No.: 101

FROM: John Doherty 8/7/87
Toxicology Branch
Hazard Evaluation Division (TS-769)

TO: Janet Andersen
Special Review Branch
Registration Division (TS-767)

THRU: Edwin Budd
Section Head
Toxicology Branch
Hazard Evaluation Division (TS-769) 8/11/87

On July 16, 1987 at 10:00 AM in the first floor conference room at East Tower (EPA facilities at Waterside Mall) a meeting was held to discuss certain toxicity studies with tributyltin chemicals. Present at the meeting were:

Joe Terek, Office of Toxic Substances, Existing Chemicals Division
Terry O'Bryan, " " " " " " "
Jim Darr " " " " " " "
Jackie Favilla, FYI Coordinator, Office of Toxic Substances, Existing Chemicals Division.
Harry Milman, Senior Science Advisor, Office of Toxic Substances.
John Doherty, Office of Pesticide Programs, Hazard Evaluation Division Toxicology Branch.

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The purpose of this meeting was for the toxicologists with interests in tributyltin compounds and other organotin compounds in the Office of Toxic Substances and the Office of Pesticide Programs to get acquainted and to discuss the available toxicity data base for these chemcials (in particular the tributyltin compounds).

Information recently provided by the WHO on a chronic feeding/oncogenicity study and special immunotoxicity testing were discussed (refer to copy of report attached). Both the representatives of the OTS and OPP agreed that the Agency should not base regulatory decisions on this summary information and that the study would have to be reviewed in its entirety before conclusions related to regulatory decisions were made. [See also the memo from Karl Baetcke, Ph.D. dated July 8, 1987, attached.]

Plans to obtain the study in its entirety were discussed. J. Doherty advised the group that at a meeting with Dr. Farber (Chief, Toxicology Branch) on June 22, 1987, it was decided that Dr. Farber would make inquires into obtaining the study data from the WHO but that at this time the results (if any) of Dr. Farber's inquiries were not known because Dr. Farber was away from his office during the week of July 13-17. The suggestion that Dr. Milman write a letter to WHO to obtain the study was considered by the group since it was Dr. Milman who obtained the preliminary information on the study. All agreed that before Dr. Milman writes such a letter, Dr. Farber would be consulted. [Refer to AFTERWARD section of this memo below]. It was generally agreed that since tributyltin is a pesticide, the study if and when it is submitted will be reviewed by Toxiology Branch.

With regard to other issues of potential toxicity of tributyltin compounds, J. Doherty presented a copy of a memo dated December 11, 1985 summarizing recognized potential toxicity problems and the data available in Toxicology Branch files on the various tributyltin compounds. Particular items discussed were:

i. Immunotoxicity. The group recognized that immunotoxicity has been identified by some investigators as the most sensitive indicator of toxicity for the organotin compounds. In their report on the chronic toxicity and carcinogenicity of bis (tri-n-butyltin)oxide in rats, Wester, Krajnc and van der Heijden maintain that available data support a NOEL of 0.5 mg/kg/day based on results of immune function studies. Refer to report from the National Institute of Public Health, Bilthoven, the Neitherlands, attached. J. Doherty stated that Toxicology Branch acknowledges this report but it is not the current position of the OPP to use this level for the purposes of ADI setting.

The chronic feeding/oncogenicity study with this chemical will have to be reviewed and it is also possible that specific immunotoxicity studies with this chemical will have to be requested to confirm the NOEL for potential immunotoxic effects.

11. Neurotoxicity. It was discussed that the tributyl tins and organotins with longer or bulkier substitute groups were not considered to be as neurotoxic as the short chain organotins such as methyl and ethyl tin. J. Doherty, however added that a recent report by J. O'Callaghan of EPA's Research Triangle Park facilities as presented at the Society of Toxicology meeting indicated that some potential effects on tributyltin derivatives on proteins in brain. In this regard, Toxicology Branch of OPP will be reevaluating the issue of potential neurotoxicity due to tributyl tins.

AFTERWARD

On Monday July 27, 1987, Dr. Farber, TB Chief, made telephone contact with the laboratory in the Netherlands concerning obtaining additional information on the chronic feeding/oncogenicity study with tributyltin in rats. Dr. Farber's contact agreed to provide a copy of the study and TB is currently waiting for this to arrive.

Attachments:

Dr. C.A. v.d. Heijen letter dated May 18, 1987 to Dr. H.A. Milman including 3 enclosures.

Dr. K.P. Baetche memo dated July 8, 1987 to Dr. Diane Beall concerning comments on the WHO chronic feeding/oncogenicity study with TBTO.

Laboratorium voor Volksgezondheid en Hygiene

220/87 Tox vH/mvr

Bilthoven, 18th May 1987

FYI-OTS-0687-0550 INIT.
SEQ. H.

H.A.Milman, Ph.D.
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U.S.A.

Dear Dr.Milman,

In answer to your letter dated 9th March 1987, I herewith send you the draft protocol and some other, perhaps interesting data regarding our chronic carcinogenicity study with organotins in rodents.

If there are any questions on this field, please don't hesitate to contact me.

Yours sincerely,

Dr.C.A.v.d.Heijden
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Toxicity of bis(tri-*n*-butyltin)oxide (TBTO) in rats

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INTRODUCTION

In view of the intended large-scale use of bis(tri-*n*-butyltin)-oxide (TBTO) as a molluscicide for the control of snails which serve as vector for Schistosoma infection in man, the WHO Parasitic Diseases Programme has requested additional data on the toxicity of TBTO. In particular, data on the long-term effects, including possible carcinogenic properties were lacking. For a summary on the toxicity of tributyltins, we refer to the previous paper (Schweinfurth, this edition).

To establish doses and relevant toxicological criteria for a combined long-term toxicity and carcinogenicity study a series of short-term studies has been carried out, the major results of which, with special emphasis on the endocrine and immune system, are presented in this paper. Part of the data has been published previously (Krajnc *et al.*, 1984; Vos *et al.*, 1984, 1985).

METHODS

General

SPF-derived Wistar rats were housed in stainless-steel cages (2 animals/cage). Tapwater and feed were supplied *ad libitum*. Experimental diets were prepared by admixing solutions of technical TBTO (purity 95.3%) in olive oil to the diet.

Animals were randomly allocated to the experimental groups. The designs of the studies are depicted in Table 1. In this paper details with regard to mitochondrial function and microsomal enzyme activities are not included. In all studies the animals remained on their diets during the whole experimental period. For a more extensive description of the methods used, see Krajnc *et al.*, 1984 and Vos *et al.*, 1984.

Endocrine function

In the additional studies involving measurement of serum hormone concentrations, animals were handled daily to minimize stress and at the end of the feeding period blood was taken by decapitation without anesthesia. Hormone-release tests were performed in cannulated rats two to three days after surgery without anesthesia.

Immune function

Mitogenic response of thymus or spleen cell suspensions was measured after incubation with phytohemagglutinin (PHA), concanavalin A (Con A), pokeweed mitogen (PWM) or lipopolysaccharide from *E.coli* (LPS). Using monoclonal antibodies against cell surface markers of T and B cells, (sub)populations were quantified with a fluorescence-activated cell sorter (FACS).

Delayed-type hypersensitivity reaction towards ovalbumin and tuberculin was studied by measuring thickness of the skin 24, 48 and 72 hr after challenge.

TBTO-exposed rats were infected orally with 1000 *T.spiralis* larvae. The yield of muscle larvae in eviscerated and skinned animals was determined after digestion 6 weeks later. Expulsion of adult worms from the small intestine was studied at day 8, 10, 12 and 14 after infection.

For determination of the humoral responses to sheep red blood cells, ovalbumin and *T.spiralis*, ELISA (IgG and IgM) and the passive anaphylaxis reaction (IgE) were used.

Gross and histopathology

At termination of the experiments, organs were weighed and sampled for histopathology. After fixation, tissues were embedded in paraplast or glycol methacrylate and cut and stained according to rou-

tine procedures. Sections from thymus, spleen, mesenteric lymph nodes, liver, thyroid and adrenals were randomized and scored blindly.

For the immunocytochemical staining (spleen, lymph nodes and pituitary) the indirect peroxidase-labelled antibody method was used.

Total tin

Total-tin measurements in feed and tissues were performed after digestion using spectrofotometric methods.

Statistical analysis

Differences in group means were tested by the Student-t test. In case of insufficient homogeneity of variances, the Welch correction with respect to the degrees of freedom was applied.

RESULTS

General toxicity

From the first week onward the animals receiving 320 mg TBTO/kg feed appeared weak and emaciated. All animals survived the feeding period. In the first week for both sexes a body weight reduction occurred at 320 mg/kg, and body weights remained constant thereafter. In the same group feed and water intakes decreased to about 50% of the control values. At 80 mg/kg the weight gain was affected slightly in males only.

Urinalysis did not reveal differences among control and treatment groups.

A dose-related increase in serum ALAT activity was present at 20 (males only), 80 or 320 mg/kg. The ASAT activity was increased in the 80 mg/kg (females) and 320 mg/kg groups (males and females). Blood glucose and liver glycogen were decreased in the 320 mg/kg group.

The insulin concentration in serum was unchanged up to 80 mg/kg. In the majority of sera from the 320 mg/kg group the insulin concentration was below the limit of detection.

IgG levels (Table 2) were reduced in the groups receiving 80 and 320 mg/kg for males and 320 mg/kg for females. For both sexes IgM levels were significantly increased at 80 and 320 mg/kg.

The leucocyte concentration (Table 3) was decreased in groups fed 80 mg/kg (males) and 320 mg/kg (males and females). Differential counts showed a marked decrease in lymphocyte concentration and increase in the concentration of neutrophils in males and females of the 320 mg/kg group, whereas the concentration of monocytes was higher from 20 mg/kg onward in males only.

Changes in hemocytometric values for both sexes (Table 4) comprised a decreased hemoglobin concentration and hematocrit value from 80 mg/kg onward. In the 20 and 80 mg/kg groups there was a slight increase in the concentration of reticulocytes. In the same group the ICDH activity was increased, whereas serum iron concentration was decreased.

The relative organ weights showed an increase over control values at 320 mg/kg for most organs. In contrast, the relative weight of the thymus was observed to decrease from 20 and 80 mg/kg for males and females, respectively.

In male rats exposed for 6 weeks no treatment-related effects were observed on the weight of the adrenals. The absolute and relative weights of the thyroid were significantly decreased at the highest dose level.

Total tin

The concentration of Sn was measured in pooled samples of kidney, liver, brain and adipose tissue. For both sexes a comparable, dose-related increase in Sn concentration was observed, the kidney and liver showing the highest levels. The ratio of Sn concentrations between subsequent dose groups was approximately 2 for the various tissues.

Endocrine function

Endocrinological aspects were studied in two additional 6-week experiments. In serum, obtained after decapitation, the concentration of Thyroxin (T4), thyroid stimulating hormone (TSH), insulin, luteinizing hormone (LH), follicle stimulating hormone (FSH) and corticosterone was determined. Insulin showed a significant decrease in both dose groups (20 and 80 mg/kg). T4 and TSH were significantly decreased at 80 mg/kg, whereas the LH concentration was increased. No statistically significant changes in FSH and corticos-

terone concentrations were apparent.

With respect to hormone release tests, no statistically significant differences were present for insulin, whereas the TSH release after TRH administration showed a tendency towards reduced secretion at 80 mg/kg. When the concentration of TSH in serum of the various groups 20 min. after thyrotropin-releasing hormone (TRH) administration was compared with the control value, a significant decrease was found. On the other hand the LH release was enhanced. A similar effect was observed for FSH.

Gross and histopathology

At necropsy the animals of the 320 mg/kg group were undersized and emaciated. Atrophy of thymus was evident; moreover, a hemorrhagic aspect was noted in the mesenteric lymph nodes, the incidence and extent of which increased in a dose-related manner in the 5 mg/kg group and higher. In the liver of three male rats in the 320 mg/kg group pale foci were observed. Except for the hemorrhagic mesenteric lymph nodes, no lesions were seen in the lower dose groups.

Histopathologic lesions observed are listed in Tables 5 and 6. No treatment-related changes were found in brain, heart, kidney, pancreas, adrenals, popliteal lymph nodes, intestinal tract and bone marrow.

Thymus. Thymic atrophy was manifest in all animals from the 320 mg/kg group, caused by lymphocyte depletion of the cortex. As result of the reduced cellularity, the cortico-medullary junctions became indistinct. Slight cortical atrophy was found in 2 male animals of the 80 mg/kg group.

Spleen. Diffuse atrophy of the white pulp was observed in the spleen of all rats of the 320 mg/kg group, affecting in particular the periarteriolar lymphocyte sheaths (PALS) and follicles. In one male and two females which had received 80 mg/kg slight atrophy was present. Perls'-stained sections showed a decrease in iron (hemosiderin) pigment in the 5 mg/kg group and higher.

Immunocytochemical staining for T-lymphocytes in the spleen revealed the presence of these cells in the PALS, and some scattered cells within the red pulp. A striking decrease in amount of immunoreactive cells was noticed in the PALS of rats of the 320 mg/kg group, corresponding with the atrophy of the PALS.

Mesenteric lymph nodes. Atrophy of the lymph nodes showed a dose related increase in incidence. Using immunocytochemistry, depletion of T-lymphocytes in the paracortex of rats of the 320 mg/kg group was noted.

The sinusoidal content of erythrocytes increased with the dose level. Red blood cells were frequently associated with mononuclear cells forming rosettes.

Liver and pancreas. In the liver of the animals from the 320 mg/kg group a marked atrophy of hepatocytes in the centrolobular areas was present, along with a decrease of glycogen content. In some animals multiple focal inflammatory processes were evident, characterized by multifocal necrosis of liver parenchyma associated with mononuclear and polymorphonuclear infiltration, fibrosis and intrahepatic bile duct hyperplasia.

In a subsequent study, attention was focussed on the liver, common bile duct and pancreas. In 2 out of 12 animals exposed to 320 mg TBTO/kg the necrotic hepatic lesions were seen at necropsy. This finding was confirmed microscopically and appeared associated with chronic ulcerative inflammation of the common bile duct. Adjacent exocrine pancreatic tissue showed only minimal involvement.

Thyroid. Flattening of the epithelial lining of thyroid follicles was observed in the 80 mg/kg group, whereas no differences were found between the 20 mg/kg group and controls after 6 weeks exposure (Table 6).

Pituitary. In the TBTO treated rats a marked decrease in number of TSH-immunoreactive cells as well as in staining intensity was seen after 6 weeks exposure to 80 mg/kg, and to a lesser degree in the 20 mg/kg group. In staining for LH, a dose-related increase was found in the number of intensely staining cells. For FSH, GH and ACTH producing cells no differences were found between controls and treated animals.

Immune function

Exposure to TBTO at 20 and 80 mg/kg feed significantly suppressed DNA synthesis in thymus cells after stimulation with PHA and PWM; the response to Con A and [^{3}H]TdR incorporation in unstimulated cultures was suppressed at 80 mg/kg.

On a cell for cell basis the response of spleen cells to PHA was significantly reduced, while the incorporation of [FiH]TdR was increased after stimulation with PWM and LPS in the 80 mg/kg group. As the number of nucleated cells was reduced, the response per whole spleen was suppressed following PHA and Con A stimulation, while PWM and LPS responses were not different between treatment and control groups.

FACS analysis indicated at 80 mg/kg a reduced percentage of T cells and a relative increase of B cells. Calculated for the whole spleen the number of B cells was comparable with that of the control group, whereas the total number of T cells was significantly reduced as well as the number of T helper and T suppressor cells.

TBTO caused a dose-related suppression of the delayed-type hypersensitivity reactions to ovalbumin and tuberculin, both at 20 and 80 mg/kg, the suppression being severe in the high-dose group.

The number of adult *T.spiralis* worms in the small intestine was measured from day 8 to 14 after infection. At day 14 the expulsion of worms was nearly complete in control animals, while expulsion was markedly impaired in the TBTO-treated ones. Counts of muscle larvae showed a significant dose-related increase. In addition, the inflammatory reaction around larva-containing muscle cells was strongly reduced at 80 mg/kg.

In the long-term immune function study, performed parallel to the chronic toxicity and carcinogenicity test, the resistance to *T.spiralis* infection was estimated after 5-6 months (in two separate tests) and 15 months. The yield of muscle larvae was significantly increased at 5 and 50 mg/kg (Table 7). In groups showing an increase in number of muscle larvae, IgE titres were reduced (Table 8).

With respect to the nonspecific resistance, TBTO treatment reduced the macrophage function as shown by the impaired splenic clearance of *Listeria monocytogenes* bacteria and reduced the activity of cytotoxic macrophages and natural killer cells.

A summary of the effects on the immune system obtained in short- and long-term studies is given in Table 9.

DISCUSSION

The toxicological profile of TBTO has been investigated in a series of short-term rat studies. In general, the target systems are in line with published data and studies presented by Schweinfurth.

Microcytic anemia suggests disturbance of hemoglobin synthesis. The tendency towards an increased fraction of reticulocytes and the enhanced ICDH activity, observed at 80 mg/kg are indicative for the presence of immature erythrocytes. Disappearance of hemosiderin in the spleen and decrease in serum iron point towards defects in iron uptake, or iron loss e.g. by mild intestinal hemorrhage.

Manabe and Wada (1981) reported that a single oral dose of triphenyltin fluoride (TPTF, 100 mg/kg body weight) to rabbits caused a.o. an increase in serum triglycerides and fasting glucose levels and inhibition of insulin release. The authors concluded that TPTF is a diabetogenic agent by inhibiting insulin release, since the pancreatic islet morphology was normal. In the present study hypoinsulinemia was found from a dietary level of 20 mg/kg onward. However, the insulin response to glucose challenge was unaltered. In addition, other data, i.e. serum glucose, ketone bodies in urine and serum triglycerides do not support a possible diabetogenic action of TBTO in the rat. The absence of measurable concentrations of insulin in most sera from animals of the 320 mg/kg-group and decreased liver glycogen may be attributed partly to the marked decrease in food intake. At lower doses the insulin concentration may reflect a decrease of the basal metabolic rate rather than a direct effect on the pancreas. The decreased T4-level is in line with this assumption.

The hepatic lesions encountered in this study in the 320 mg/kg group included necrotic areas with an exudative and proliferative inflammatory response, associated with ulcerative inflammation of the common bile duct. Similar findings are reported by Barnes and Magee (1958) after single or repeated high doses of di-*n*-butyltin dichloride in the rat, and it seems justified to conclude that the liver lesions found in the 320 mg/kg group are primarily caused by toxicity for the bile duct system.

An other prominent feature encountered was the presence of erythrocyte rosettes in the mesenteric lymph nodes. The mechanism of rosette formation is unclear: the occurrence of erythrocytes may be

attributed to micro-hemorrhages in the intestinal tract drained by these lymph nodes. However, such hemorrhages could not be demonstrated histopathologically. Rosette-arrangements might be due to deficient phagocytosis.

Reduced serum thyroxin and TSH levels, as well as decreased activity of the thyroid at microscopic examination have also been reported earlier by Funahashi *et al.* (1980). However, in contrast with their findings a marked decrease in immunoreactivity for TSH in the pituitary was noted, which is consistent with reduced serum TSH values. Equally conflicting with the findings of Funahashi *et al.* (1980), after subacute and semichronic exposure, no effect was seen on ACTH-producing cells in the anterior pituitary and on pars intermedia and adrenal histology. Serum corticosterone levels and adrenal weight were unaffected. Thus, it can be concluded that 6-week feeding at the 80 mg/kg level does not result in stimulation of the pituitary-adrenal axis. With respect to the gonadotropins in TBTO-exposed rats, an increase was found in the number of pituitary cells staining intensely for LH, while no effect was observed on FSH-producing cells. These results correlate well with data obtained by radioimunnoassay.

As expected, a number of parameters points towards an effect of TBTO on the immune system. Thymic atrophy, observed at 20 (males), 80 and 320 mg/kg, is reported earlier after three week feeding of tri-*n*-butyltin chloride (150 mg/kg) to weanling rats (Seinen and Penninks, 1979). Leucocytopenia is also observed in mice (Ishaaya *et al.*, 1976). The increase in serum IgM and decrease in IgG levels could suggest an impairment in the function of T-helper cells, since IgG antibody synthesis is thought to be more thymus-dependent than the IgM synthesis. From the various immune function studies carried out with TBTO in weanling male rats, both in short- and long-term tests it is concluded that this chemical causes a pronounced suppression of the thymus-dependent immunity as shown by depressed delayed-type hypersensitivity reactions, suppressed resistance to *T.spiralis* and reduced response of thymus and spleen cells to T-cell mitogens. The latter phenomenon is explained by reduced numbers of T-cells as shown by surface marker analysis. Regarding the thymus-dependent antibody response, IgE titres to *T.spiralis* are suppressed, while IgM and IgG antibody responses to *T.spiralis* ovalbumin and tetanus toxoid are not impaired. In addition, the mononuclear phagocyte system is compromised

Table 4. Hematological and biochemical parameters in male rats after dietary TBTO exposure for 6 weeks[a]

Dietary concentration (mg/kg)	0	5	20	80
hemoglobin (mmol/l)	10.1	10.0	9.8	9.1[b]
hematocrit (l/l)	0.42	0.41	0.40[c]	0.37[b]
erythrocytes (10^{12}/l)	7.7	7.6	7.8	8.0
thrombocytes (10^{12}/l)	0.82	0.84	0.88	0.97
reticulocytes (10^{12}/l)	0.38	0.36	0.43	0.44
ICDH (U/10^{12} ery)	30.3	29.5	30.6	34.6[c]
Fe (mmol/l)	44.5	40.0	42.5	32.3[c]

[a] From Krajnc et al., 1984.
Values are means of 9-10 animals/group. Abbreviations: ICDH, isocitrate dehydrogenase activity; Fe, serum iron concentration

[b] $P < 0.001$

[c] $0.01 \leq P < 0.05$

as shown by decreased *in vivo* splenic clearance of *L.monocytogenes*. With respect to the natural cell-mediated cytotoxicity, dietary exposure to TBTO reduces the activity of NK cells in the spleen and of cytotoxic macrophages in the peritoneal cavity.

CONCLUSIONS

TBTO-induced thymic atrophy and suppression of the thymus-dependent immunity are explained by a direct toxic action of this pesticide on thymic lymphocytes. In addition, pronounced effects were found on the pituitary-thyroid and pituitary-gonad axis.

For the long-term toxicity and carcinogenicity study dose levels of 0.5, 5 and 50 mg TBTO/kg feed are expected to be adequate to estimate a no-adverse effect level.

In view of the action on gonadotropic hormones, further studies with respect to reproduction are indicated.

Data on long-term alterations in immune and endocrine function are essential for risk assessment.

ACKNOWLEDGMENTS

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Table 1. Experimental design of studies performed with TBTO

RANGE-FINDING EXPERIMENT

Animals	: Wistar Riv:TOX(S-C), 10 males and females/group 110-130 g (age 5-6 weeks)
Exposure	: 4-week continuous oral administration via the diet
Dose levels	: 0, 5, 20, 80 and 320 mg TBTO/kg feed
Examinations	: symptoms, food and water consumption, urinalysis, biochemistry, hematology, immunoglobulins, organ weights, macroscopy, histopathology, total tin in tissues

ADDITIONAL SHORT-TERM STUDIES

Animals	: Wistar Riv:TOX (S-C), 8-10 males/group 40-60 g (age 3-4 weeks)
Exposure	: 6-week continuous oral administration via the diet
Dose levels	: 0, 20 and 80 mg TBTO/kg feed
Objectives	: - hematology - endocrine function - histopathology/immunocytochemistry - immune function - mitochondrial function - microsomal enzyme activities

LONG-TERM EXPERIMENT

Animals	: Wistar Riv:TOX(S-C), 50 males/group 40-60 g (age 3-4 weeks)
Exposure	: 6-17 months continuous oral administration via the diet
Dose levels	: 0, 0.5, 5 and 50 mg TBTO/kg feed
Objectives	: thymus-dependent and nonspecific immunity and host resistance

Table 2. IgM and IgG levels in serum of rats after dietary TBTO exposure for 4 weeks[a]

Dietary concentration (mg/kg)	IgM %	IgG %
MALES		
0	100 ± 22	100 ± 34
5	99 ± 26	118 ± 46
20	100 ± 17	96 ± 42
80	132 ± 20[c]	61 ± 30[b]
320	223 ± 86[c]	39 ± 12[d]
FEMALES		
0	100 ± 14	100 ± 28
5	94 ± 19	110 ± 45
20	113 ± 17	107 ± 54
80	145 ± 30[d]	90 ± 45
320	151 ± 27[d]	30 ± 12[d]

[a] From Krajnc et al., 1984. $\bar{x}$ ± SD of 10 animals per group as percentage of control

[b] $0.01 \leq P < 0.05$

[c] $0.001 \leq P < 0.01$

[d] $P < 0.001$

Table 3. White blood cell counts in rats after dietary TBTO exposure for 4 weeks[a]

Dietary concentration (mg/kg)	0	5	20	80	320
MALES					
leucocytes (10^6/l)	12360	12480	11420	10500[b]	7080[c]
eosinophils	67	24[d]	72	30[d]	18[b]
basophils	12	7	5	17	7
neutropnils	821	965	735	916	1815[b]
lymphocytes	11186	11057	10109	9045[b]	4753[c]
monocytes	274	427	499[d]	491[d]	487[d]
FEMALES					
leucocytes (10^6/l)	9840	8780	9140	9380	5960[c]
eosinophils	63	74	62	37	19[d]
basophils	15	11	0	5	6
neutrophils	687	774	887	1082	1262[b]
lymphocytes	8761	7521	7871	7837	4349[c]
monocytes	314	400	320	419	324

[a] From Krajnc et al., 1984. Values are means of 10 animals/group

[b] $0.001 \leq P < 0.01$

[c] $P < 0.001$

[d] $0.01 \leq P < 0.05$

Table 5. Incidence of histopathologic lesions in rats after dietary TBTO exposure for 4 weeks[a]

Dietary concentration (mg/kg)	0		5		20		80		320	
	m	f	m	f	m	f	m	f	m	f
THYMUS examined	10	10					10	10	10	10
atrophy cortex slight							2			
marked									10	10
SPLEEN examined	10	10	10	10	10	10	10	10	10	10
atroph white pulp slight							1	2	6	4
marked									3	6
hemosiderin content deplete			2		5		10	1	5	
low	4	1	4		5	3		5	5	3
moderate	5	2	4	5		6		4		5
high	1	7		5		1				2
MESENTERIC LYMPH NODE examined	10	10	10	10	10	10	10	10	10	10
atrophy slight		1			2	3	4	2	3	1
moderate									5	4
marked									2	5
erythrocyte rosettes few-moderate	1		7	2	5	3	2	1	4	1
abundant					3	2	8	9	5	9
LIVER examined	10	10					10	10	10	10
atrophy hepatocytes slight								3	3	
marked									6	10
bile duct hyperplasia									4	1
necrotic areas									3	

[a] From Krajnc *et al.*, 1984.

No treatment-related lesions were observed in brain, kidney and heart after 4 weeks, and in pancreas, adrenals, popliteal lymph nodes, gastrointestinal tract and bone marrow after 6 weeks; for effects on thyroid and pituitary see Table 6.

Table 6. Incidence of histopathologic lesions in thyroid and immunocytochemistry of pituitary of male rats after dietary TBTO exposure for 6 weeks[a]

Dietary concentration (mg/kg)	0	20	80
THYROID examined	6	6	6
activity normal	5	6	
slight decrease	1		3
marked decrease			3
PITUITARY examined	6	6	5-6
immunoreactivity for TSH normal	5		
slight decrease	1	4	2
marked decrease		2	3
immunoreactivity for LH normal	5	3	2
increase	1	3	4

[a] From Krajnc et al., 1984.

No differences were observed between control and 80 mg/kg group with respect to immunoreactivity for follicle stimulating hormone (FSH), growth hormone (GH) and adrenocorticotropic hormone (ACTH) in the pituitary

Table 7. Resistance to *Trichinella* infection in male rats after short-t and long-term TBTO exposure

Dietary concentration (mg/kg)	6 weeks[a]	5.5 months Exp. 1	5.5 months Exp. 2	16.5 mo
0	74 ± 22[b]	30 ± 25	32 ± 21	34
0.5		40 ± 21	42 ± 22	33
5		59 ± 21[d]	67 ± 36[d]	53
20	106 ± 20[c]			
50		99 ± 26[e]	98 ± 24[e]	138
80	198 ± 34[e]			

[a] From Vos *et al.*, 1984.

[b] Count of muscle larvae (10^3/carcass); $\bar{x}$ ± SD of 9 - 12 animals/group

[c] $0.01 \leq P < 0.05$

[d] $0.001 \leq P < 0.01$

[e] $P < 0.001$

Table 8. Resistance to *Trichinella* infection (serum IgE titres) in male rats after short-term and long-term TBTO exposure

Dietary concentration (mg/kg)	6 weeks	6 months	17 months
0	3.8 ± 1.2	4.6 ± 2.0	3.8 ± 2.0
0.5		2.9 ± 2.6	3.2 ± 1.9
5		2.8 ± 1.6[b]	1.9 ± 1.6[c]
20	2.5 ± 1.7[b]		
50		1.7 ± 2.1[c]	2.0 ± 2.1[b]
80	1.8 ± 0.8[d]		

[a] From Vos *et al.*, 1984.
IgE titrations were performed by passive cutaneous anaphylaxis reactions in sera, 3 weeks after infection. Values (x ± SD of 9-12 animals/group) are expressed as the ^{2}log antibody titre.

[b] $0.01 \leq P < 0.05$

[c] $0.001 \leq P < 0.01$

[d] $P < 0.001$

Table 9. Summary of functional assessment of specific and nonspecific resistance after short-term and long-term exposure in the rat

	6 weeks[a]		4-6 months			15-17 months		
Dietary concentration (mg/kg)	20	80	0.5	5	50	0.5	5	50
Thymus-dependent immunity								
T.spiralis infection: expulsion of adult worms	↓	↓↓						
resistance to muscle larvae	↓	↓↓	-	↓	↓↓	-	↓	↓↓
IgE response	↓	↓	-	↓	↓↓	-	↓	↓
Delayed type hypersensitivity to ovalbumin and tuberculin	↓	↓↓	-	-	-	-	-	-
IgM and IgG response to *T.spiralis* and ovalbumin	-	-	-	-	-	-	-	-
IgG response to sheep red blood cells	-	↓				-	-	-
T-mitogen response, thymus	↓	↓↓	-	-	↓			
T-mitogen response, spleen	-	↓	-	-	-	-	-	-
Nonspecific resistance								
L.monocytogenes infection, splenic clearance	↓	↓↓	-	-	↓	-	-	↓
Natural cell-mediated cytotoxicity, peritoneal macrophages	↓	↓	-	-	-			
Natural cell-mediated cytotoxicity, splenic NK cells	-	↓	-	-	↓	↓	↓	↓

[a] From Vos *et al.*, 1984.

- no suppression; ↓ slight to moderate suppression; ↓↓ strong suppression

NATIONAL INSTITUTE OF PUBLIC HEALTH.
BILTHOVEN, THE NETHERLANDS.

Protocol for a lifetime carcinogenicity and toxicity study of the Molluscicide Tributyltinoxide (TBTO) in rats.

At the request of the World Health Organization. Geneva, Switzerland.

Introduction

At the request of WHO on behalf of the Parasitic Disease Programme (PDP) a lifetime carcinogenicity and toxicity study will be carried out in rats wit the molluscicide Tributyltinoxide (TBTO).

The objective of the study is to investigate the possible carcinogenic potential of the test-compound, to detect long term adverse-effects and to establish a possible no-effect level in rats.

Initially a 4-week range-finding study will be carried out to determine proper dose-levels for the long term study.

During the range-finding study, special attention will be given to the palatability of the food, effects of the compound on possible target organs: liver, kidneys, lymphoid organs and brain, accumulation of tin in these organs and excretion of tin with urine.

1. PERSONEL

A. SCIENTISTS.

Dr.C.A.van der Heijden. Pathologist.
Head Laboratory of Carcinogenesis and Mutagenesis.

Dr.E.I.Krajnc. Toxicologist.
Head Section on Metal Toxicology.
Laboratory of General Toxicology.

Dr.H.A.M.G.Vaessen. Analytical chemist.
Laboratory of Chemical Analysis of Foods.

Dr.P.W.Wester. Pathologist.
Section on Oncology and Patholgy of Toxic Compounds.
Laboratory of Pathology.

B. STUDY DIRECTOR.

Dr.C.A.van der Heijden.

C. TECHNICAL STAFF.

A competent and experienced tecnnical staff from the various cooperating laboratories will carry out their operations under the direct supervision of the above mentioned scientists.

2. TESTSUBSTANCE

Technical TBTO of known source and (im)purity, characterized by the manufacturer for the entire study of the same batch, will be provided by M & T. The test-compound will be stored accordingly to written instructions of the manufacturer and analysed at regular intervals during the study to check stability.
Before starting the long term study, the stability of TBTO in the diet will be determined during a period of 2 weeks at least.

3. ANIMAL SPECIES

Laboratories own breeding colony, SPF derived; strain identification: rat Riv: TOX [M].

4. ROUTE OF ADMINISTRATION

TBTO will be administered to the rats orally. The compound will be preferab incorporated into the diet. Preliminary work will be carried out to test stabilit and adsorption of TBTO in the diet to ensure that the animals are actually exposed to the test-compound. The alternative is to mix TBTO into the drinking-water; this is considered less suitable because of the low solubility of TBTO. Relevant exposure levels cannot be obtained and disturbances in water intake will produce serious difficulties in the evaluation of the results. The compound is easily adsorbed to the wall of drinking bottles.

If, as a result from stability tests that TBTO seems to decompose rapidly i diet, it might be desirable to administer the compound by gavage.

5. RANGE-FINDING STUDY

This experiment will involve 4 groups of each 10 male and 10 female weanlin rats. Growth and food-intake will be recorded weekly. At week 4 limited clinical chemistry and haematology will be performed. Pathology will be restricted to gross changes at autopsy and histopathology of brain*, liver*, spleen*, thymus*, kidneys* and mesenteric lymphe nodes of control and high dose group. Treatment related pathology will be examined at all dose levels. In addition serum IgG and IgM will be determined. Total tin contents will be determined in 5 male and 5 fem rats per group in liver, kidney, brain, spleen, fat and blood.

*organ weights measured

6. DOSE SELECTION

The study will consist of 4 experimental groups: 1 control and 3 treatment groups. A positive control group will not be included. Selection of the high dose will be based on short term toxicity-data of TBTO, which will be provided by the manufacturer and the results of a 4 weeks-range-finding study, wh has the objective to establish proper dose levels and to test the response of the strain of rats used for the long term study.

The high dose level should produce some signs of toxicity without shortenir the lifespan of the rats; whereas the low dose would allow to establish a no-effect level. The intermediate dose will be scaled from the high dose by a factor 5 or 10.

7. DURATION OF THE EXPERIMENT

The study will start at weaning age and exposure to the test compound is continued during at least two years. The study will be terminated when mortality reaches 60% or after two and half years.

At 12 month 10 male and 10 female rats will be killed to examine e.g. haema tology, clinical chemistry, organ weights, (histo)pathology and tin accumulation o tin of tin in target organs.

8. NUMBER OF ANIMALS PER GROUP

Each experimental group consists of 60 male and 60 female rats. In addition 6 male and 6 female rats/animal room will be added to the experiment for the serological monitoring of possible infectious diseases, which might interfere with the study. (See experimental scheme).

experimental group	males	females
negative control group	60	60
low dose group	60	60
intermediate dose group	60	60
high dose group	60	60
infections disease monitory group	12	12

9. METHODS OF RANDOMIZATION

The animals will be allocated at random to their experimental group and experimental animal number. Individual weights and other relevant individual data, if available, as date of birth and distribution of litter mates will be recorded. The rats will be housed in two identical rooms at two per cage.

The cages of each experimental group will be blocked together in one of the rooms. Ill or dead animals will be replaced only during the first two weeks of the study.

10. ANIMAL HUSBANDRY

The SPF-bred animals will be kept under clean, but conventional conditions, in wire-mesh, stainless-steel cages. Individual animals per cage will be identified by earmark. The animal rooms are well ventilated, having slightly reduced pressure, and controlled lighting, temperature and humidity. Access to the animal rooms will be restricted to experienced personnel essential to the study in question.

Early detection and check for infectious diseases will be facilitated by serological testing, at regular intervals, of a "monitor" group of rats added to the study.

11. DIETS

The animals will be fed commercial animal food according to the Laboratory's Standard procedure. (Composition known).

At regular intervals the diets will be analysed for the presence of contaminants such as residues of certain pesticides chlorinated hydrocarbons, nitro compounds, mycotoxins (aflatoxin B_1, G_1 and M_1). and heavy metals. The results of these analyses will be incorporated in the final report.

11. SAFETY MEASURES

According to the Laboratory's Standard procedure routine precautions will be taken to prevent inadvertent exposure of personnel and the environment to the test substance. Control measurements will be carried out in the animal and diet preparation room.

During preparation of the diet and animal handling biotechnicians will use a disposable mask, or depending on results of the above mentioned analyses

a pressurised air mask, to prevent inhalatory exposition to the test-compound. Because TBTO is rapidly reduced to inorganic tin, no special measurements will be taken with regard to disposal of contaminated waste, including faeces, dead bodies and spilled food. To exclude possible mutagenicity, which render the proposed safety measures less valid, the compound will be tested in the Ames-test before starting the long term study.

12. OBSERVATIONS

a. Clinical signs and mortality.

Physical examination for general health and tumour development of each animal will be conducted weekly. For mortality the cages will be checked daily (after 1 year twice daily). Positive findings will be recorded on the individual observation sheets, attached to the cages of the animals.

b. Bodyweight, food and water consumption.

Bodyweight, food and water consumption will be recorded weekly during the first 12 weeks and thereafter every four weeks.

c. Analysis of TBTO in feed.

TBTO in the diet will be analysed at regular intervals. Depending on the frequency of preparing fresh TBTO/food mixtures. (See pt. 2 Test substance).

d. Haematology and clinical chemistry.

At 12 months and at termination of the study haematology and clinical chemistry will be carried out on 10 male and 10 female rats of each group.
The following measurements will be made: haematology: haemaglobin and haematocrit values, total erythocyte and leucocyte counts, and differentiated white blood cell counts. Clinical chemistry: SGPT/SGOT, urea, glucose, BSP/PSP and creatinine. Urinalysis: semi-quantative urinalysis; protein, creatinine: quantitatively, and osmolality and concentration test. Other measurements may be included depending on the results from the range-finding study.
Total tin contents will be determined depending on the results from the range-finding study in liver, kidney, brain, spleen, fat and blood at 12 months and at the end of the study from 5 males and 5 females per group.

e. Pathology.

Gross pathology will be performed on 1) all animals dying intercurrently or being killed because of ill health, and 2), on the animals being killed at 12 months and at termination of the study. All macroscopic findings will

be recorded on an individual sheet which is also a checklist to ensure, that all organs are inspected and collected.

Histopathology for non-neoplastic changes will be carried out on 10 animals of either sex of control and high dose group, killed at 12 months and of 20 male and 20 female rats killed at the end of the study (± 25 organs e.g. tissue samples per animal). Non-neoplastic histopathological changes, which are considered treatment-related, will be studied subsequently in the lower dose groups.

To begin with the histological search for tumours, it will be restricted to all surviving and non-surviving animals from control- and high dose group. From these animals all tissue samples, collected at autopsy, and all lesions grossly suspected of being tumourous, will be studied histologically for cancerous and preneoplastic changes. If indicated, the collected tissue samples of the lower dose groups will be studied histologically for neoplastic changes.

From all animals the following organs will be taken at autopsy: brain*, heart*, lungs, liver*, spleen*, kidneys*, pituitary, thyroids (attached to trachea), thymus (when visible), pancreas, adrenals*, ovaries, testes*, uterus, prostate, mesenterical lymph nodes, salivary glands, stomach, oesophagus, duodenum, jejunum, ileum, coecum, colon, rectum, urinary bladder, vertebral column, spinal cord, ischiadic nerve and striated muscle (musc. quadriceps). From the G.I.-tract Swiss rolls will be prepared. Lungs and urinary bladder will be inflated with formaline at autopsy.

Data acquisition, processing and storage, including storage of specimen and raw data will be carried out in accordance with the principles of Good Laboratory Practice and Laboratory Standard procedures.

*organ weights: organs taken at autopsy at 12 months and at the end of the study marked with an asterisk * will be weighed.

Tentative evaluation of TBTO for human safety

Bis(tri-n-butyltin)oxide (TBTO) has been tested at the NIPHEH with respect to its general toxicity and its effect on the immune system.

As already documented for a number of organotin compounds, TBTO appears to suppress the immune system, being the most sensitive criterion in rats after oral exposure. A clear dose-effect relationship could be demonstrated. In addition, comparable results were obtained in experiments with exposure periods varying between 6 weeks and 15 months, i.e. no aggravation is observed with increasing exposure time.

From function tests and mechanistic studies it is concluded that TBTO reduces cellular immune responses by exerting toxicity towards the immature thymocyte, reducing equally T helper and T suppressor cell populations in the periphery. Also parameters of nonspecific immunity are affected as shown by decreased activity of natural killer cells and macrophages.

Immune suppression by chemicals may give rise to opportunistic infections and may enhance cancer risk in experimental animals and man. Current knowledge of chemically induced immune suppression indicates a dose-response relation showing a threshold and reversibility of the effects. In contrast, a viral etiology (AIDS), although showing similarities, i.e. suppression of the thymus-dependent immunity and similar endpoints, exhibits also marked differences, e.g. specific target is the T helper cell population. Moreover, host defense progressively declines in AIDS patients.

Since human toxicity data are lacking, a risk evaluation of TBTO can only be based on data from animal experiments. The negative results obtained in a battery of mutagenicity tests and the results of a carcinogenicity study in rats justify in our view a standard threshold-based extrapolation to arrive at a provisional acceptable daily intake for humans. A long-term feeding study in rats indicates a "no toxic effect level" of 0.5 mg TBTO/kg feed based on immunological criteria. Using a safety factor of 100, ingestion of 15 ug TBTO/day may be regarded as acceptable for a 60 kg person. Assuming that fish consumption constitutes the only source of TBTO and that 100 g of fish is eaten daily, 150 ug TBTO/kg in fish would be acceptable.

In view of the presence of TBT-compounds in the aquatic environment and the known bioaccumulation and high toxicity for several aquatic species, it is essential that the risks for the environment should be included in the evaluation of the use of TBTO.

CHRONIC TOXICITY AND CARCINOGENICITY STUDY WITH BIS(TRI-N-BUTYLTIN)OXIDE (TBTO) IN RATS

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INTRODUCTION

For the safety evaluation of bis(tri-n-butyltin)oxide (TBTO) to be used as molluscicide, a chronic toxicity and carcinogenicity study in rats was requested by the World Health Organization (Parasitic Disease Programme); this study was conducted at the National Institute of Public Health and Environmental Hygiene in the Netherlands. At the time of this workshop, the histopathological data of this study were preliminary regarding the examination of the intermediate dose groups for several neoplastic and non-neoplastic changes, as well as some statistical analyses to be done.

METHODS

The study was started with 4 groups, each consisting of 60 SPF-derived weanling Wistar rats (Riv:TOX) of either sex, 10 of which were allocated for chronic toxicity monitoring after one year. The dose range in the feed was 0, 0.5, 5, and 50 mg/kg, based upon the results of the previous range finding study (Krajnc et al., 1984; Krajnc et al., this volume). The termination of the experiment was scheduled at 30 months or 60% mortality, at which time point also 10 animals per sex and group were allotted for toxicity screening.

The following parameters were monitored:

General: mortality; body weight, food consumption; water intake (during the whole experiment).

Clinical chemistry (plasma): ALAT; ASAT; alkaline phosphatase; creatinine kinase; glucose; urea; IgG; IgM (after 3, 12 and 24 months).

Urinalysis: osmolality and volume; protein concentration; creatinine clearance (after 3, 12 and 24 months).

Hematology: hemoglobin; hematocrit; MCV; MCH; MCHC; total erythrocyte count; total and differential leukocyte counts; platelet count (after 3, 12 and 24 months).

Endocrinology: insulin; TSH; LH; FSH; T4, both free and bound (after 3, 12 and 24 months).

Total tin: in liver and kidney (after 12 and 24 months).

Organ weights: at necropsy at 12 and 24 months, 10 animals per sex and group.

Gross pathology: all animals after scheduled and unscheduled sacrifice.

Histopathology: all tissues (28 per animal) collected at scheduled sacrifice for nonneoplastic lesions; all tissues (28 standard samples and in addition, all tissues suspected for tumors) from all animals from top dose and control groups for (pre)neoplastic lesions; intermediate dose groups for (pre)neoplastic lesions from organs affected in high dose group.

RESULTS

For both sexes, mortality increased significantly towards the end of the study due to age-related conditions. This was significantly higher in the high dose group of both males and females and reached the 60% level in males at week 106, at which point the experiment was terminated. The mortality curves are shown in Fig.1A and 1B.

During the second experimental year, body weight decreased in the males of the high dose group, and the females in this group showed less body weight gain (Fig. 2). Food consumption was higher in the 5 mg/kg

groups, especially in males; in the 0.5 mg/kg group increased food consumption was seen only during a limited period, and in the 50 mg/kg group food intake was in the same range as in the controls. Water intake was increased in a dose-related manner in the 5 and 50 mg/kg groups after the first half year, and was also more pronounced in males.

In clinical chemistry, the major findings were an increase in IgM and decrease in IgG in the females only of the 50 mg/kg group, and an increase in alkaline phosphatase in both sexes.

Renal function was not significantly affected, as in urinalysis only a slightly reduced concentration capacity was found in the females of the 50 mg/kg group after two years.

Hematological examination showed a reduced number of peripheral blood lymphocytes and an increase in platelets in the high dose females. However, these changes were found after one year only.

In contrast to the short-term studies, no major effects were found in endocrinological parameters; only the FT4/T4 ratio was lower in both sexes, although the absolute levels were statistically unchanged.

Tin contents of liver and kidney increased slightly in a dose-related manner, up to 5-10 mg/kg organ weight in the high dose groups. Differences between 1 and 2 year only did occur in the female kidney, showing highest concentrations after 2 year.

Histopathological examination after 1 year showed a reduced liver glycogen and spleen iron content in the 50 mg/kg groups, and flattening of the thyroid epithelium. After 2 years the lower epithelium in the thyroid was still present, and there was an increase in age-related degenerative kidney lesions.

Tumor incidences were significantly increased in the anterior pituitary (prolactinomas, as demonstrated by immunohistochemical staining) in the 0.5 and 50 mg/kg groups of both sexes (Table 1). This was mainly due to an increase in fatal tumors, which were qualified as such by their large

volume (up to 1 cm in diameter) and cerebral compression with the subsequent severe central nervous impairment or death. Tumors of the adrenal medulla (pheochromocytomas) were markedly increased in both sexes of the 50 mg/kg group, whereas in males only, adrenal cortical adenomas were decreased (Table 2). Also in males only, parathyroid adenomas were increased. In the females, two poorly differentiated adenocarcinomas of pancreatic origin with generalized metastases were observed in the high dose group and one in the lowest dose group.

A summary of the main results is listed in Table 3.

DISCUSSION

Lifetime feeding of TBTO to rats showed toxic effects of dietary levels of 5 mg/kg and higher. Signs of toxicity occurred later in the course of the study, however, without specific target organ involvement. With respect to neoplastic diseases, changes (notably increases) occurred at the 50 mg/kg feeding level in some common age-related benign tumors in endocrine-sensitive organs which also have shown high spontaneous levels in our strain of rats (Kroes et al., 1981; Wester et al., 1985a; 1985b). The number of pituitary tumors showed, without a clear dose-response relationship, only a marginal increase in the lowest dose group (0.5 mg/kg). Because of the absence of a dose-relationship and the high and variable spontaneous incidences of this type of tumor in the strain of rats used, the increased incidence in the low dose groups is considered fortuitous and not related to treatment with TBTO. Most findings seem consistent with aging, and therefore it can be speculated that long-term low dose feeding with TBTO enhances the process of aging, or that the incidence of the changes may be influenced by endocrine - metabolic interference or immunomodulation, as suggested from the results from this and previous studies (Krajnc et al., 1984; Vos et al., 1984; 1985; Krajnc et al., this volume). The occasional occurrence of adenocarcinomas of the

exocrine pancre although not dose-related and not statistically significant, are of interest as this type of tumor is rare in our strain of rats (Kroes et. al., 1981, Wester et al., 1985a; 1985b) and high-grade malignant, as seen by their generalized metastatic spread. It is suggested that the toxic effect of TBTO and DBTC on the bileduct system, although at high concentrations (Krajnc et al., 1984; Barnes and Magee, 1958), might play a role in the pathogenesis of the pancreatic lesion, as this tissue is intimately connected with the bileduct (Barnes and Magee, 1958). Mainly based on the results of the immune function studies, reported in the previous paper (Krajnc et al., this issue) it is concluded that the no-toxic effect level for lifetime feeding in rats is 0.5 mg/kg diet. The results of this study can not be considered as evidence for carcinogenicity. However, the tumors in the exocrine pancreas, as well as the increase in incidence of some common tumors in endocrine tissues, warrant a long-term study in a second species.

ACKNOWLEDGEMENTS

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Table 1

INCIDENCE OF PITUITARY TUMORS IN RATS AFTER LIFETIME EXPOSURE TO TBTO

	FEMALES		MALES	
mg/kg	total tumors	fatal tumors	total tumors	fatal tumors
0	22	3	34	5
0.5	32*	5	39*	11*
5	22	4	29	9
50	35**	11**	43***	17***

Statistical analysis according to Peto (1980), one-tailed.

*: $p < 0.05$

**: $p < 0.01$

***: $p < 0.001$

Table 2

INCIDENCE OF ADRENAL TUMORS IN RATS AFTER LIFETIME EXPOSURE TO TBTO

	FEMALES		MALES	
mg/kg	cortex	medulla	cortex	medulla
0	8	3	13	16
0.5	7	3	5	13
5	8	3	11	14
50	8	34***	2**	33***

Statistical analysis according to Peto (1980), one-tailed.

**: $p < 0.01$

***: $p < 0.001$

Table 3

SUMMARY OF MAIN FINDINGS IN RATS AFTER CHRONIC EXPOSURE TO TBTO

parameter	effect	sex		conc. TBTO (mg/kg)
Mortality	↑	F	M	50
Body weight	↓	F	M	50
Food consumption	↑		M	5
Water intake	↑	F	M	5, 50
Serum alk.phosp.	↑	F	M	50
Serum ALAT, ASAT	↑	F	M	50
Serum urea	↓	F	M	50
IgM	↑	F		50
IgG	↓	F		50
Lymphocytes*	↓	F		[5, 50]
FT4/T4	↓	F	M	50
Urine conc.	↓	F		50
Adrenal weight	↑	F	M	50
Liver glycogen*	↓	F	M	50
Bileduct hyperpl.*	↑	F	M	50
Spleen iron*	↓	F	M	50
Thyroid activity	↓	F	M	50
Nephrosis	↑	F	M	50
Pituitary Tumors	↑	F	M	0.5, 50
Pheochromocytomas	↑	F	M	50
Adr.Cort.Tumors	↓		M	50
Carcinoma Pancreas	↑	F		0.5, 50
Adenoma Parathyroid	↑		M	50

*: effect observed after 1 year only.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: FYI-OTS-0687-0550-INIT; SEQA 84-870000132; Review of Long-Term Bioassays

FROM: Karl P. Baetcke, Ph.D.
Acting Section Chief
Oncology Section
Health and Environmental
Review Division (TS-796)

TO: ~~Jackquely~~ Jacqueline Favilla
FYI Coordinator
Chemical Screening Branch
Existing Chemical
Assessment Division (TS-778)

THRU: Diane D. Beal, Ph.D.
Chief
Oncology Branch
Health and Environmental
Review Division (TS-796)

The subject document was reviewed for adequacy of carcinogenicity data from a long-term bioassay of tributyl tin oxide (TBTO) in rats. The information provided in the report is insufficient to determine if TBTO evoked a carcinogenic response in the rat bioassay, although the limited information provided suggests a positive reponse. Information lacking in the report that precludes an evaluation of carcinogenic potential includes, but is not limited to:

1. Tables 1 and 2 furnish information on incidences of total tumors. It is unclear if the numbers given are percentages, if more than one tumor per animal was reported, or how many animals were found to have tumors versus animals at risk.

2. Although historical rates of commonly occuring or rare tumors are used as a basis to reach various conclusions in the discussion, the historical rates are not given. Consequently, an independent evaluation of the significance of tumor incidences can not be made.

3. The authors conclude in the Discussion section that "this study can not be considered as evidence for carcinogenicity." The basis for this conclusion, that the findings of tumors appeared to be fortiutous because of high background rates and lack of a dose respnse, may be inconsistent with EPA hazard assessment procedures. The occurence of multiple tumor types, increases in rare tumors, and appearance of tumors in both sexes suggests carcinogenic potential of TBTO was demonstrated.

It is recommended that a complete copy of the final report on the long-term bioassay of TBTO be requested in order that a full evaluation of the carcinogenic potential of the chemical can be made.